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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Tumor invasion requires destruction of collagen, and is accomplished by matrix metalloproteinase-1 (MMP-1). A single nucleotide polymorphism (SNP) in the MMP-1 promoter enhances transcription of this gene. The SNP is at -1607 bp in the MMP-1 promoter, where an additional guanine (G) creates a binding site (5 -AGGA-3) for the Ets transcription factors. Allele frequency is: 25% =1 G homozygous, 25% =2 G homozygous, and 50% = 1G/2G heterozygous. We hypothesized that the 2G SNP was associated with aggressive breast cancer. MMP-1 is on chromosome 11q22.2-22.3, a region associated with Loss of Heterozygosity (LOH) in breast cancer, and that retaining the 2G allele after LOH provided tumors with an advantage for progression. Therefore, we (1) genotyped DNA from normal tissues and metastatic breast tumors for the SNP (2) evaluated tumors for LOH, and (3) measured MMP-1 mRNA levels. Of the 58 individuals genotyped, 24 were heterozygous, and of these only 5 underwent LOH, with 3 retaining the 2G allele and 2 retaining the 1G allele. mRNA analysis of tissues from 45 breast cancer patients revealed substantial MMP-1 mRNA expression in 32. Thus, although LOH may not favor the 2G allele, MMP-1 mRNA expression is common in breast cancer. MMP-1 may be a marker for women at risk for invasive/metastatic breast cancer.

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breast cancer invasion/metastasis, matrix metalloproteinase 1 mRNA, single nucleotide polymorphism (SNP), loss of heterozygosity

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Introduction

Degradation of the extracellular matrix is the *sine qua non* of tumor invasion and metastasis, and it is mediated primarily by matrix metalloproteinases (MMPs). Destruction of the interstitial collagens, types I and III, is a necessary part of this process, since these collagens comprise nearly 30% of body protein and make up the connective tissues through which tumor cells must travel during invasion. Collagen degradation is accomplished primarily by a sub-group of MMPs, the collagenases. Of the three interstitial collagenases that can contribute to invasion, MMP-1 (collagenase-1) is the most ubiquitously expressed and thus, may have the greatest potential for facilitating tumor invasion. We have found a single nucleotide polymorphism (SNP) in the MMP-1 promoter that greatly enhances transcription of this gene in tumor cells and in normal stromal cells, thereby potentially facilitating cancer progression by more aggressive degradation of the interstitial matrix by either the tumor cells or the neighboring stromal cells. The single nucleotide polymorphism (SNP) is located at -1607 bp in the MMP-1 promoter, where an additional guanine (G) creates a site (5'-AGGA-3'), which binds members of the Ets family of transcription factors, and the absence of the G (5'-AGA-3') lacks this site. The two alleles at this locus, 1G and 2G, are present at approximately equal frequency in normal populations, with 25% being 1G/1G homozygous, 50% being 1G/2G heterozygotes, and 25% being 2G/2G homozygotes. Importantly, the prevalence of the 2G allele was increased significantly in breast tumor cell lines compared to the population gene frequency (P= 0.0001), suggesting a potential role of this SNP in invasive breast cancer. Because MMP-1 expression in breast cancer correlates with an aggressive phenotype, the 2G genotype may serve as a marker for invasive and aggressive disease.

Furthermore, analysis of DNA taken from both normal tissues and from metastatic tumor indicates that some breast cancers display loss of heterozygosity (LOH) at 11q22-23, the chromosomal location of the MMP-1 gene. We have hypothesized that retention of the 2G allele would be associated with aggressive disease, suggesting that these metastatic tumors may have a selective advantage over those with the 1G allele, and that increased expression of MMP-1 may be associated with tumor progression. Thus, we have a structural variation in the MMP-1 gene that may be a useful genetic marker in breast cancer and that is easily detected.

Accordingly, the specific aims of this study are:

1. To genotype DNA from a spectrum of breast tumors (primary and metastatic, ductal carcinoma in situ (DCIS); invasive ductal; lobular; inflammatory) in order to evaluate the frequency of the MMP-1 SNP in the different types and stages of breast cancer (I – IV).

2. To evaluate, within breast tumor types, Loss of Heterozygosity (LOH) according to stage of disease. To this end, normal and tumor tissues from the same patient will be analyzed to determine LOH in the tumor.

3. To examine breast cancer tissues by immunohistochemistry and in situ hybridization for expression of MMP-1 protein and mRNA. These studies will determine whether it is the stromal cells and/or the tumor cells that are contributing to the invasive behavior of the tumors, and will be correlated with results of genotyping.

These studies may lead to a new approach for predicting the invasive potential of breast tumors, and may influence the choice of therapies for treating specific breast cancers that contain this variation.

Body

Statement of Work and Progress towards Stated Tasks

* denotes task for year 1

** denotes tasks for year 2

Aim/Task 1: *To genotype DNA from a spectrum of breast tumors (primary and metastatic; ductal carcinoma in situ [DCIS]; invasive ductal; lobular; inflammatory) in order to evaluate the frequency of the MMP-1 SNP in the different types and stages of breast cancer (I – IV).*

* (1). Months 1- 9: Begin initial review of pathology reports on breast cancer specimens housed at the Dartmouth Hitchcock Medical Center. Sort records into type of breast cancer, e.g. primary, metastatic, invasive ductal, lobular, inflammatory, ER +/-. Based on review of records, retrieve pathology slide for examination by a pathologist, who will delineate tumor tissue on the slide. Cut additional slides from block of fixed tissue. ALMOST COMPLETED

* (2). Months 3 - 9: Begin extraction of tumor tissues from tissue blocks, and perform PCR amplification for the 1G vs 2G MMP-1 SNP. Whenever possible, amplify normal tissue from the same individual. ON GOING

** (3). Months 9 - 18: Continue with review of tissue samples. Continue amplification for the MMP-1 SNP in tumor and normal tissue. Begin to analyze data to correlate the 1G vs 2G SNP with tumor type, primary or metastatic disease, etc. ON GOING

** Months 18 - 24: Examine data to determine where additional samples are needed in order to substantiate previous findings. ON GOING

Months 26 - 36: Finish examination of patient records, PCR amplification of normal and tumor tissues and correlation of SNP with clinical status.

Aim/Task 2: *To evaluate, within breast tumor types, the presence of LOH according to stage of disease. To this end, normal and tumor tissues from the same patient will be analyzed.*

* Months 1- 12: Genotype normal tissue as well as breast tissue from the same individual. Begin to compare the genotype of breast tumors with that of normal tissue taken from the same individual. ON GOING

* *Months 12 -18: Begin to analyze genotype of tumor tissue vs. normal tissue for LOH. ON GOING

Months 18 -24 : Continue analysis of tumor and normal tissue for LOH in tumors. Where LOH has occurred, begin mapping studies to delineate the boundaries of LOH. ON GOING

Months 24 -36: Finish assaying samples for LOH, finish mapping region of LOH. Correlate findings with type of breast cancer and state of disease.

Aim/Task 3: *To examine breast cancer tissues by immunohistochemistry and in situ hybridization for expression of MMP-1 protein and mRNA. These studies will determine whether it is the stromal cells and/or the tumor cells that are contributing to the invasive behavior of the tumors, and results will be correlated with genotyping.*

** Months 6 - 24: Examine tissue samples from patients for expression of MMP-1 protein/mRNA. ON GOING

** Months 18 - 24: Begin analysis of data to determine if additional samples are needed from the same or other individuals in order to validate findings. ON GOING

Months 24 – 36: Finish immunohistochemistry and in situ hybridization. Correlate these expression data with analysis of the 1G vs 2G SNP and with LOH.

Accomplishments: As noted in the proposal, we have identified a single nucleotide polymorphism (SNP) in the promoter of the MMP-1 gene that increases its expression (1). This SNP is the insertion/deletion of an extra guanine (G), so as to create the sequence 5'-GGAA-3' (2G allele) vs. 5'-GAA-3'(1G allele). The increase in MMP-1 expression mediated by the 2G allele may increase collagen degradation, thereby potentiating tumor invasion. Thus, the overall goal of this study is to evaluate the role of the 2G allele in the invasive behavior of breast cancer. In year one of this project, we began our analysis with invasive ductal carcinoma because it is the most common form of breast cancer. Further, based on our previous experiences with breast cancer cell lines and metastatic melanoma, we initially examined metastatic breast tumors derived from regional lymph nodes. We are in the process of analyzing 95 cases of invasive metastatic ductal carcinoma. Analysis has been completed on 65. Slides from an additional eight specimens have been dissected by Laser Capture Microscopy, 20 more slides are being cut and two more cases have been identified recently. Slides will be ordered on these. In summary, of the 95 cases identified to date, two-thirds (65/95) have been studied for the 1G/2G genotype and LOH. Over the coming year, we will complete the analysis of the remaining 30 cases, and identify and examine five additional cases, bringing the total for this portion of the study to 100 cases.

As described in the proposal, we are examining tumor tissue from the heterozygotes for LOH at the 11q 22-23 locus, a common site for LOH in breast cancer and the location of the MMP-1 gene (2-4). We are using our 32P PCR assay with overlapping sets of primers (82 bp or 72 bp) (2) to amplify DNA from the tumor tissue (2). Of the 24 heterozygotes detected so far, we have observed LOH in only 5 (Figure 1), suggesting that LOH at this locus may not be as common an event in breast cancer as previously reported (3,4). Of these five, three have retained the 2G allele and two have retained the 1G allele. Note the reproducibility in data derived from both the 72 bp and the 82 bp amplicon. Note also that the degree of LOH in replicates from any one patient is remarkably consistent and ranges from about 25% to 35% 2G in those two samples retaining the 1G allele, and 60% to 80% 2G in samples retaining the 2G allele. These findings validate our quantitative assay for LOH (2) and substantiate our data. However, the number of cases analyzed so far appears to be too small to permit valid conclusions about the potential selective advantage of retaining the 2G allele compared to the 1G allele. Thus, we plan to analyze additional samples.

In those patients whose metastatic tumor displayed LOH, we examined the primary tumor from those individuals to determine if LOH was an early event, occurring at the site of the primary tumor. Two primary tumors showed no LOH, two showed LOH with retention of the 2G allele, and one showed retention of the 1G allele. Thus, when LOH does occur at this locus, it appears to be an early event in that it is present in the primary tumor (Figure 1). Again, the degree of LOH in the primary and in metastatic tumors from any individual remained consistent, suggesting that tumors may contain a component of normal stromal tissue (2).

In addition to these studies, which were designed to analyze the SNP in breast cancer *progression*, we examined the association of the 2G allele with breast cancer *incidence*. The 1G/2G genotype of 157 women was evaluated by PCR of DNA obtained by buccal swabs. Of these, 82 were invasive breast cancer cases representing ductal, lobular, and ductal with a lobular component, and 75 were from normal controls. Genotypes of women with cancer were: 29% = 1G/1G, 21% = 2G/2G and 50% = 1G/2G, and genotypes of control women were: 27% =1G/1G, 27% = 2G/2G and 47% = 1G/2G, indicating no link between the 2G allele and cancer incidence. These findings contrast with those of Kanamori (5), Nishioka (6) and Zhu (7), who demonstrated a significant correlation

between the 2G allele and the incidence of ovarian, endometrial and lung cancer, respectively.

Finally, we are beginning an analysis of MMP-1 mRNA expression in normal and tumor tissues at various stages of tumor progression. Samples were taken from 45 women, in addition to those 95 mentioned above for LOH analysis. The samples represent normal tissue, atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC). Samples were graded histopathologically on a scale of I to III by a pathologist for the stage of disease for both DCIS and IDC. Samples were prepared from frozen sections, dissected with the Laser Capture Microscope, and MMP-1 mRNA was amplified by real-time RT-PCR for 40 cycles. The raw data from these studies are submitted in Appendix I. There is an enormous amount of information to be derived from them, and we stress that we are in the earliest stages of data analysis. Relative MMP-1 expression can be determined by comparing the threshold cycle (C_t) for normal tissue to that of tumor tissue. The threshold cycle is defined as the point where fluorescence of an unknown sample moves above background. This value is inversely proportional to the amount of input mRNA of the specific target, in this case MMP-1. Therefore, a C_t=40 means the fluorescence never gets above background and there is no target mRNA present. A lower C_t indicates larger amounts of target mRNA.

Of the 45 individuals examined, 32 (71%) expressed substantial levels of MMP-1 mRNA, and in 25 of these individuals, this expression was often seen in the very early stages of disease, i.e., at ADH or DCIS. Further, fold change in mRNA levels was calculated as the increase over normal control tissue and expressed as $\log_2$, since MMP-1 DNA standards were diluted 2 fold, from 1:4 to 1:256. In general, MMP-1 mRNA increased with the disease progression (Table 1), with grade I tumors showing lower levels of MMP-1 compared to grade II and grade II showing lower levels compared to grade III. (Raw data are presented as Appendix II). These findings suggest that MMP-1 expression is common in breast cancers, even at the early stages of disease, and that expression increases as tumors progress. We are in the process of genotyping these specimens so that we can correlate the level of MMP-1 gene expression with the 1G vs. 2G genotype. These expression studies will be confirmed with immunohistochemistry.

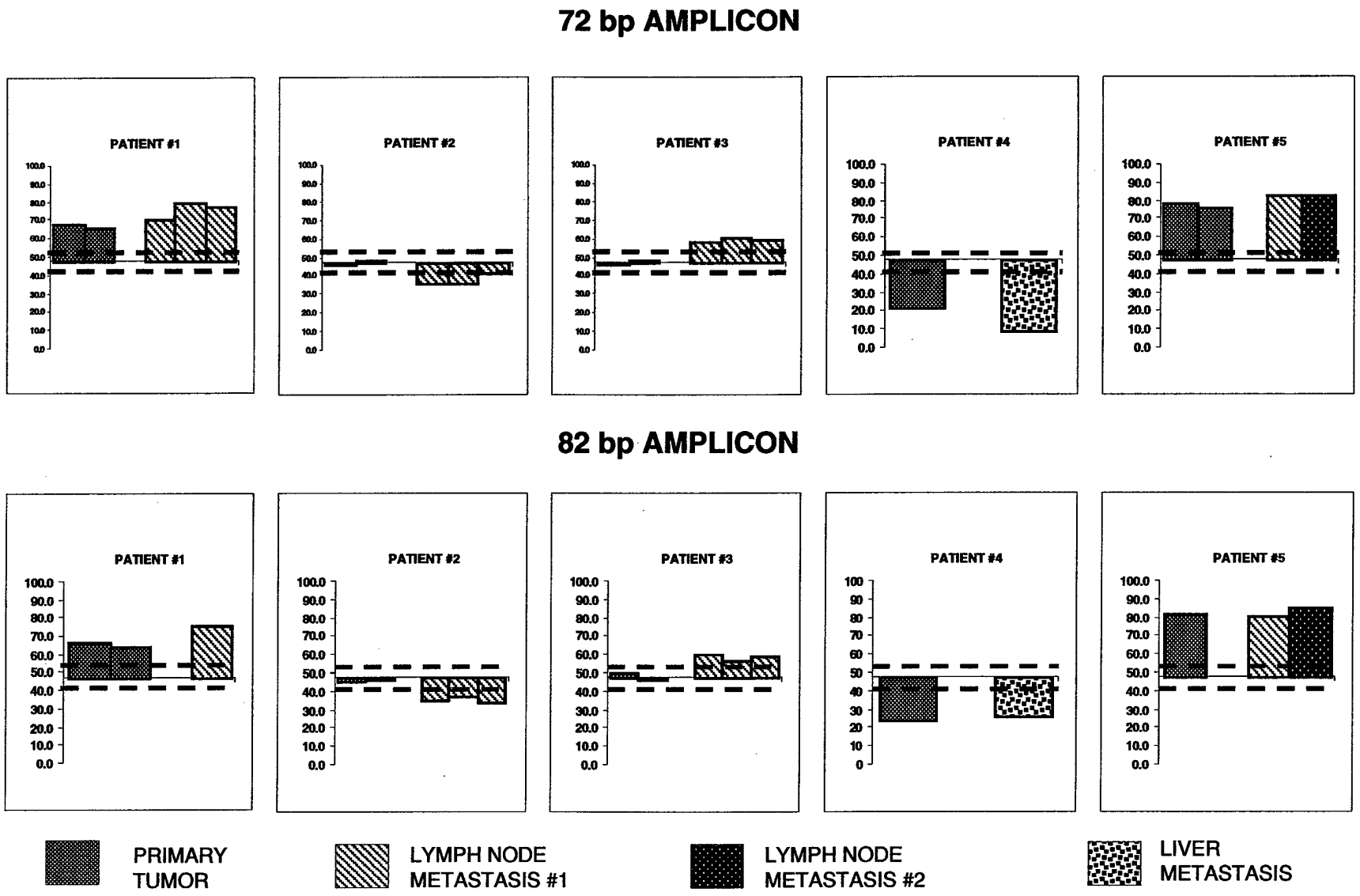

FIGURE 1: LOH analysis of replicate primary and metastatic tumor samples in which LOH was detected. Each bar represents a separate laser capture microdissection sample. The upper and lower parts of the figure display the data obtained with the 72 and 82 bp primer sets, respectively. The y-axis represents the % 2G allele in the sample. The x-axis crosses the y-axis at 47.4 % 2G for both the 72 and 82 bp primers, and represents the mean of multiple (N=84 for 72 bp primers, N=81 for the 82 bp primers) samples collected from normal breast tissue from heterozygous study subjects; the dotted lines denote +/-2SD for these data. LOH was considered to be present if replicate analyses of tumor exceeded +/-2SD for both the 72 and 82 bp amplicons. Patient 1 has LOH with retention of the 2G allele in both the primary tumor and in a lymph node metastasis. Patient 2 has LOH with retention of the 1G allele in a lymph node metastasis but not in the portion of the primary tumor that was examined. Patient 3 has LOH with retention of the 2G allele in the lymph node metatstasis but not in the portion of the primary tumor that was examined. Patient 4 has LOH with retention of the 1G allele in both the primary tumor and a liver metastasis. Patient 5 has LOH with retention of the 2G allele in the primary tumor and in two separate lymph node metastases.

Table 1. Fold increase in MMP-1 mRNA correlated with grade of breast cancer in DCIS and IDC.

Grade	Fold Increase	Stand Dev	SEM
I	320.8	762.0	288.0
II	4368.9	10729.1	3576.4
III	34054.3	86164.7	28721.6

Key Research Accomplishments

- Identified and retrieved 95 specimens from metastatic ductal carcinoma
- Cut histologic sections on 73 of these, with 20 additional in progress, two more cases identified and five more anticipated.
- Dissected normal and breast tissue from 65 of these
- Genotyped normal and breast tissue from these
- Examined 24 heterozygotes for LOH
- Detected LOH in 5/24: retention of the 2G allele in 3 samples, retention of the 1G allele in 2 samples.

Reportable Outcomes

¥ Abstract submitted to DoD, April 1, 2002

- manuscript on the role of the SNP in breast cancer incidence and progression is in preparation, and awaits analysis of the final samples before completion.
- manuscript anticipated on MMP-1 expression levels and correlation with 1G/2G genotype and mRNA expression. These studies will be confirmed by immunohistochemistry to localize MMP-1 to the tumor cells and/or surrounding stromal tissues.

Conclusions

1. The study is progressing well and on time.

2. The data obtained so far support the hypothesis that the 2G allele does not predispose women to breast cancer. However, we do not yet know whether it is associated with progression of established disease. We do know that LOH at the 11q22-23 locus is relatively rare. Further, the fact that each allele seems to be retained with the same frequency may imply that there is no selective pressure to retain the 2G allele.

3. The data obtained so far indicate that MMP-1 expression is a common and early event in breast cancer. Further, the levels of expression increase as the disease progresses from ADH to DCIS to IDC.

4. The "so what" of this study are the facts that (a) the 2G allele does not seem to predispose Caucasian women to breast cancer, a finding that contrasts with studies on ovarian (5), endometrial (6) and lung (7) cancers, and (b) MMP-1 mRNA is expressed by breast cancer tissues. As the studies continue, and we correlate expression with the SNP genotype, we may find that the 2G allele does not mediate increased MMP-1 expression, and that compensatory mechanisms are present to drive MMP-1 expression in cells containing either the 1G or the 2G allele. This is similar to our studies on MMP-1 expression in on a melanoma cell line, which is homozygous for the 1G allele, but which expresses high levels of MMP-1 (8). *The findings derived from breast cancer are important in understanding the pathogenesis of breast cancer and in designing therapies to prevent metastasis.*

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Appendices

Appendix I: real-time RT-PCR for MMP-1 in breast cancer tissues

Appendix II: fold increase in MMP-1 expression in grade I, II and III breast cancers.

Appendix III: DoD abstract for September, 2002 meeting.

APPENDIX I: REAL-TIME RT-PCR FOR MMP-1 in BREAST CANCER TISSUES

MMP1 #1

			TARGET							Normalizer (Cyclophilin, 18S)						
			Sample	Ct	Mean Ct	St. Dev.	ng	Avg. ng	St.Dev. ng	Ct	Mean Ct	St. Dev.	ng	Avg. ng	St.Dev. ng	
A		1	Stock 1:4	20.1578	20.17	0.02	192.88	190.83	2.27	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A		2	Stock 1:4	20.1709			191.24			0.00			#DIV/0!			
A		3	Stock 1:4	20.194			188.39			0.00			#DIV/0!			
A		4	Stock 1:16	22.0522	22.12	0.08	56.28	53.97	2.65	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A		5	Stock 1:16	22.2012			51.08			0.00			#DIV/0!			
A		6	Stock 1:16	22.1003			54.55			0.00			#DIV/0!			
A		7	Stock 1:64	24.3934	24.38	0.06	12.28	12.38	0.51	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A		8	Stock 1:64	24.4393			11.92			0.00			#DIV/0!			
A		9	Stock 1:64	24.3145			12.93			0.00			#DIV/0!			
A		10	Stock 1:256	26.5574	26.53	0.03	3.01	3.07	0.06	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A		11	Stock 1:256	26.4992			3.12			0.00			#DIV/0!			
A		12	Stock 1:256	26.524			3.07			0.00			#DIV/0!			
B		1	130N	40	40.00	0.00		0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	35.01
B		2	130N	40			0.00			0.00			10571499726.70			
B		3	130N	40			0.00			0.00			10571499726.70			
B		4	130DCIS	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
B		5	130DCIS	40			0.00			0.00			10571499726.70			
B		6	130DCIS	40			0.00			0.00			10571499726.70			
B		7	130IDC	40	40.00	0.00		0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	38.13
B		8	130IDC	40			0.00			0.00			10571499726.70			
B		9	130IDC	40			0.00			0.00			10571499726.70			
B		10	131N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
B		11	131N	40			0.00			0.00			10571499726.70			
B		12	131N	40						0.00			10571499726.70			37.28
C		1	131ADH	33.7474	33.96	0.20	0.03	0.02	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C		2	131ADH	33.9819			0.02			0.00			10571499726.70			
C		3	131ADH	34.1515			0.02			0.00			10571499726.70			
C		4	131DCIS	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C		5	131DCIS	40			0.00			0.00			10571499726.70			
C		6	131DCIS	40			0.00			0.00			10571499726.70			
C		7	131IDC	32.2195	32.15	0.08	0.08	0.08	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C		8	131IDC	32.0662			0.08			0.00			10571499726.70			
C		9	131IDC	32.1718			0.08			0.00			10571499726.70			
C		10	133N	37.7498	38.57	0.73	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C		11	133N	38.8264			0.00			0.00			10571499726.70			
C		12	133N	39.1332			0.00			0.00			10571499726.70			
D		1	133DCIS	24.4943	24.35	0.16	11.50	12.68	1.33	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D		2	133DCIS	24.3766			12.42			0.00			10571499726.70			
D		3	133DCIS	24.1783			14.13			0.00			10571499726.70			
D		4	133IDC	28.0043	28.06	0.05	1.17	1.13	0.03	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D		5	133IDC	28.0792			1.12			0.00			10571499726.70			
D		6	133IDC	28.0881			1.11			0.00			10571499726.70			
D		7	148N	35.6362	35.33	0.29	0.01	0.01	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D		8	148N	35.2917			0.01			0.00			10571499726.70			
D		9	148N	35.0547			0.01			0.00			10571499726.70			
D		10	148DCIS	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D		11	148DCIS	40			0.00			0.00			10571499726.70			
D		12	148DCIS	40			0.00			0.00			10571499726.70			
E		1	148IDC	32.6254	32.66	0.24	0.06	0.06	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E		2	148IDC	32.9075			0.05			0.00			10571499726.70			
E		3	148IDC	32.4343			0.07			0.00			10571499726.70			
E		4	152N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E		5	152N	40			0.00			0.00			10571499726.70			
E		6	152N	40.00						0.00			10571499726.70			34.11
E		7	152DCIS	28.7895	28.93	0.12	0.70	0.65	0.05	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E		8	152DCIS	28.9772			0.62			0.00			10571499726.70			
E		9	152DCIS	29.0091			0.61			0.00			10571499726.70			
E		10	153N	31.4117	31.28	0.13	0.13	0.13	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E		11	153N	31.16						0.00			10571499726.70			33.47
E		12	153N	31.2725			0.14			0.00			10571499726.70			
F		1	153IDC	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F		2	153IDC	40			0.00			0.00			10571499726.70			
F		3	153IDC	40			0.00			0.00			10571499726.70			
F		4	169N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F		5	169N	40			0.00			0.00			10571499726.70			
F		6	169N	40			0.00			0.00			10571499726.70			
F		7	169DCIS	27.1476	27.20	0.06	2.05	1.98	0.08	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F		8	169DCIS	27.1962			1.98			0.00			10571499726.70			
F		9	169DCIS	27.2643			1.90			0.00			10571499726.70			
F		10	169IDC	25.2557	25.38	0.11	7.01	6.47	0.47	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F		11	169IDC	25.4409			6.22			0.00			10571499726.70			
F		12	169IDC	25.4512			6.17			0.00			10571499726.70			
G		1	180N	36.2243	35.88	0.39	0.01	0.01	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G		2	180N	35.4526			0.01			0.00			10571499726.70			
G		3	180N	35.964			0.01			0.00			10571499726.70			
G		4	180ADH	31.6114	31.53	0.08	0.11	0.12	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G		5	180ADH	31.5257			0.12			0.00			10571499726.70			
G		6	180ADH	31.4617			0.12			0.00			10571499726.70			
G		7	180DCIS	31.6391	31.43	0.18	0.11	0.13	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G		8	180DCIS	31.3412			0.13			0.00			10571499726.70			
G		9	180DCIS	31.3131			0.14			0.00			10571499726.70			
G		10	180IDC	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G		11	180IDC	40			0.00			0.00			10571499726.70			
G		12	180IDC	40			0.00			0.00			10571499726.70			
H		1	183N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H		2	183N	40			0.00			0.00			10571499726.70			
H		3	183N	40			0.00			0.00			10571499726.70			
H		4	183DCIS	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H		5	183DCIS	40			0.00			0.00			10571499726.70			
H		6	183DCIS	40			0.00			0.00			10571499726.70			
H		7	184N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H		8	184N	40			0.00			0.00			10571499726.70			
H		9	184N	40			0.00			0.00			10571499726.70			
H		10	184DCIS	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H		11	184DCIS	40			0.00			0.00			10571499726.70			
H		12	184DCIS	40			0.00			0.00			10571499726.70			

MMP142

			TARGET						Normalizer (Cyclophilin, 18S)							
			Sample	Ct	Mean Ct	St. Dev.	ng	Avg. ng	St.Dev. ng	Ct	Mean Ct	St. Dev.	ng	Avg. ng	St.Dev. ng	
A		1	Stock 1:4	21.2968	21.25	0.04	188.62	194.97	5.54	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A		2	Stock 1:4	21.2269			197.40			0.00			#DIV/0!			
A		3	Stock 1:4	21.2154			198.88			0.00			#DIV/0!			
A		4	Stock 1:16	23.3143	23.30	0.01	50.74	51.17	0.42	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A		5	Stock 1:16	23.3005			51.20			0.00			#DIV/0!			
A		6	Stock 1:16	23.2894			51.57			0.00			#DIV/0!			
A		7	Stock 1:64	25.4492	25.42	0.03	12.65	12.89	0.22	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A		8	Stock 1:64	25.4106			12.97			0.00			#DIV/0!			
A		9	Stock 1:64	25.3994			13.06			0.00			#DIV/0!			
A		10	Stock 1:256	27.6249	27.64	0.04	3.07	3.04	0.07	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A		11	Stock 1:256	27.6164			3.09			0.00			#DIV/0!			
A		12	Stock 1:256	27.6816			2.96			0.00			#DIV/0!			
B		1	5N	40.00	40.00	0.00		0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	35.01
B		2	5N	40			0.00			0.00			10571499726.70			
B		3	5N	40			0.00			0.00			10571499726.70			
B		4	5DCIS	32.7546	32.65	0.14	0.11	0.12	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
B		5	5DCIS	32.4893			0.13			0.00			10571499726.70			
B		6	5DCIS	32.7072			0.11			0.00			10571499726.70			
B		7	5IDC	40.00	40.00	0.00		0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	38.13
B		8	5IDC	40			0.00			0.00			10571499726.70			
B		9	5IDC	40			0.00			0.00			10571499726.70			
B		10	14N	37.4212	37.31	0.40	0.01	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
B		11	14N	37.8418			0.00			0.00			10571499726.70			
B		12	14N	36.86						0.00			10571499726.70			37.28
C		1	14DCIS	33.7459	33.59	0.14	0.06	0.06	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C		2	14DCIS	33.4607			0.07			0.00			10571499726.70			
C		3	14DCIS	33.5726			0.06			0.00			10571499726.70			
C		4	14IDC	32.7669	32.67	0.09	0.11	0.12	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C		5	14IDC	32.6504			0.12			0.00			10571499726.70			
C		6	14IDC	32.5812			0.12			0.00			10571499726.70			
C		7	30N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C		8	30N	40			0.00			0.00			10571499726.70			
C		9	30N	40			0.00			0.00			10571499726.70			
C		10	30DCIS	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C		11	30DCIS	40			0.00			0.00			10571499726.70			
C		12	30DCIS	40			0.00			0.00			10571499726.70			
D		1	30IDC	25.8159	25.81	0.05	9.96	9.99	0.32	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D		2	30IDC	25.8599			9.68			0.00			10571499726.70			
D		3	30IDC	25.7608			10.33			0.00			10571499726.70			
D		4	41N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D		5	41N	40			0.00			0.00			10571499726.70			
D		6	41N	40			0.00			0.00			10571499726.70			
D		7	41DCIS	32.2936	32.29	0.07	0.15	0.15	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D		8	41DCIS	32.3547			0.14			0.00			10571499726.70			
D		9	41DCIS	32.218			0.15			0.00			10571499726.70			
D		10	41IDC	33.1492	32.93	0.21	0.08	0.10	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D		11	41IDC	32.7234			0.11			0.00			10571499726.70			
D		12	41IDC	32.9323			0.10			0.00			10571499726.70			
E		1	43N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E		2	43N	40			0.00			0.00			10571499726.70			
E		3	43N	40			0.00			0.00			10571499726.70			
E		4	43DCIS	31.3976	31.46	0.05	0.26	0.26	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E		5	43DCIS	31.4653			0.25			0.00			10571499726.70			
E		6	43DCIS	31.51						0.00			10571499726.70			34.11
E		7	43IDC	29.8204	29.81	0.04	0.74	0.74	0.02	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E		8	43IDC	29.7625			0.76			0.00			10571499726.70			
E		9	43IDC	29.8434			0.72			0.00			10571499726.70			
E		10	44N	36.8955	37.18	0.29	0.01	0.01	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E		11	44N	37.17						0.00			10571499726.70			33.47
E		12	44N	37.4739			0.01			0.00			10571499726.70			
F		1	44DCIS	36.3896	36.89	0.52	0.01	0.01	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F		2	44DCIS	36.8559			0.01			0.00			10571499726.70			
F		3	44DCIS	37.4368			0.01			0.00			10571499726.70			
F		4	44IDC	29.8088	29.89	0.11	0.74	0.71	0.05	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F		5	44IDC	30.0091			0.65			0.00			10571499726.70			
F		6	44IDC	29.841			0.73			0.00			10571499726.70			
F		7	57N	37.2562	37.06	0.26	0.01	0.01	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F		8	57N	37.1627			0.01			0.00			10571499726.70			
F		9	57N	36.7696			0.01			0.00			10571499726.70			
F		10	57ADH	33.8906	34.02	0.12	0.05	0.05	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F		11	57ADH	34.1265			0.04			0.00			10571499726.70			
F		12	57ADH	34.0302			0.05			0.00			10571499726.70			
G		1	57DCIS	31.8834	31.90	0.12	0.19	0.19	0.02	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G		2	57DCIS	32.033			0.17			0.00			10571499726.70			
G		3	57DCIS	31.7869			0.20			0.00			10571499726.70			
G		4	65N	34.8519	34.86	0.03	0.03	0.03	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G		5	65N	34.8409			0.03			0.00			10571499726.70			
G		6	65N	34.8978			0.03			0.00			10571499726.70			
G		7	65DCIS	34.1062	33.88	0.25	0.05	0.05	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G		8	65DCIS	33.9371			0.05			0.00			10571499726.70			
G		9	65DCIS	33.8059			0.06			0.00			10571499726.70			
G		10	65IDC	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G		11	65IDC	40			0.00			0.00			10571499726.70			
G		12	65IDC	40			0.00			0.00			10571499726.70			
H		1	72N	32.5657	32.56	0.02	0.12	0.12	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H		2	72N	32.5733			0.12			0.00			10571499726.70			
H		3	72N	32.5372			0.13			0.00			10571499726.70			
H		4	72DCIS	33.0781	33.02	0.06	0.09	0.09	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H		5	72DCIS	32.9613			0.10			0.00			10571499726.70			
H		6	72DCIS	33.0081			0.09			0.00			10571499726.70			
H		7	75N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H		8	75N	40			0.00			0.00			10571499726.70			
H		9	75N	40			0.00			0.00			10571499726.70			
H		10	75DCIS	35.608	35.71	0.22	0.02	0.02	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H		11	75DCIS	35.5593			0.02			0.00			10571499726.70			
H		12	75DCIS	35.9633			0.01			0.00			10571499726.70			

MMP1 #3

			TARGET						Normalizer (Cyclophilin, 18S)							
		Sample	Ct	Mean Ct	St. Dev.	ng	Avg. ng	St.Dev. ng	Ct	Mean Ct	St. Dev.	ng	Avg. ng	St.Dev. ng	
A	1	Stock 1:4	20.1001	20.06	0.08	189.17	194.88	9.72	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A	2	Stock 1:4	20.0985			189.37			0.00			#DIV/0!			
A	3	Stock 1:4	19.9668			206.10			0.00			#DIV/0!			
A	4	Stock 1:16	22.1858	22.12	0.07	49.49	51.73	2.51	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A	5	Stock 1:16	22.1315			51.25			0.00			#DIV/0!			
A	6	Stock 1:16	22.0377			54.43			0.00			#DIV/0!			
A	7	Stock 1:64	24.3812	24.30	0.09	12.07	12.69	0.74	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A	8	Stock 1:64	24.3274			12.49			0.00			#DIV/0!			
A	9	Stock 1:64	24.2053			13.51			0.00			#DIV/0!			
A	10	Stock 1:256	26.5551	26.51	0.12	2.98	3.07	0.24	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A	11	Stock 1:256	26.6057			2.89			0.00			#DIV/0!			
A	12	Stock 1:256	26.3819			3.33			0.00			#DIV/0!			
B	1	45N	33.06	33.30	0.31		0.04	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	35.01
B	2	45N	33.6486			0.03			0.00			10571499726.70			
B	3	45N	33.1921			0.04			0.00			10571499726.70			
B	4	45DCIS	31.0015	30.88	0.14	0.17	0.19	0.02	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
B	5	45DCIS	30.9028			0.18			0.00			10571499726.70			
B	6	45DCIS	30.73			0.20			0.00			10571499726.70			
B	7	60N	40.00	39.93	0.12		0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	38.13
B	8	60N	40			0.00			0.00			10571499726.70			
B	9	60N	39.7968			0.00			0.00			10571499726.70			
B	10	60DCIS	34.0647	34.41	0.54	0.02	0.02	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
B	11	60DCIS	34.1319			0.02			0.00			10571499726.70			
B	12	60DCIS	35.03						0.00			10571499726.70			37.28
C	1	79 N	33.6648	33.44	0.20	0.03	0.04	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C	2	79 N	33.3868			0.04			0.00			10571499726.70			
C	3	79 N	33.2786			0.04			0.00			10571499726.70			
C	4	79 ADH	39.0334	39.68	0.56	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C	5	79 ADH	40			0.00			0.00			10571499726.70			
C	6	79 ADH	40			0.00			0.00			10571499726.70			
C	7	79 DCIS	40	39.70	0.52	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C	8	79 DCIS	40			0.00			0.00			10571499726.70			
C	9	79 DCIS	39.0946			0.00			0.00			10571499726.70			
C	10	79 IDC	33.1813	32.99	0.32	0.04	0.05	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C	11	79 IDC	32.6135			0.06			0.00			10571499726.70			
C	12	79 IDC	33.1613			0.04			0.00			10571499726.70			
D	1	88 N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D	2	88 N	40			0.00			0.00			10571499726.70			
D	3	88 N	40			0.00			0.00			10571499726.70			
D	4	88 DCIS	24.4002	24.33	0.06	11.92	12.45	0.47	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D	5	88 DCIS	24.3112			12.62			0.00			10571499726.70			
D	6	88 DCIS	24.287			12.82			0.00			10571499726.70			
D	7	88 IDC	22.2677	22.21	0.06	46.95	48.84	2.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D	8	88 IDC	22.1407			50.95			0.00			10571499726.70			
D	9	88 IDC	22.2132			48.63			0.00			10571499726.70			
D	10	89 N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D	11	89 N	40			0.00			0.00			10571499726.70			
D	12	89 N	40			0.00			0.00			10571499726.70			
E	1	89 DCIS	33.437	33.40	0.11	0.04	0.04	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E	2	89 DCIS	33.474			0.03			0.00			10571499726.70			
E	3	89 DCIS	33.2761			0.04			0.00			10571499726.70			
E	4	96 N	39.0489	39.44	0.50	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E	5	96 N	39.2789			0.00			0.00			10571499726.70			
E	6	96 N	40.00						0.00			10571499726.70			34.11
E	7	96 DCIS	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E	8	96 DCIS	40			0.00			0.00			10571499726.70			
E	9	96 DCIS	40			0.00			0.00			10571499726.70			
E	10	96 IDC	37.237	37.49	0.29	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E	11	96 IDC	37.42						0.00			10571499726.70			33.47
E	12	96 IDC	37.8029			0.00			0.00			10571499726.70			
F	1	102 N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F	2	102 N	40			0.00			0.00			10571499726.70			
F	3	102 N	40			0.00			0.00			10571499726.70			
F	4	102 DCIS	32.1719	31.96	0.23	0.08	0.09	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F	5	102 DCIS	31.7231			0.11			0.00			10571499726.70			
F	6	102 DCIS	32.0537			0.09			0.00			10571499726.70			
F	7	102 IDC	33.4595	33.59	0.39	0.04	0.03	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F	8	102 IDC	33.2814			0.04			0.00			10571499726.70			
F	9	102 IDC	34.0212			0.02			0.00			10571499726.70			
F	10	112 N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F	11	112 N	40			0.00			0.00			10571499726.70			
F	12	112 N	40			0.00			0.00			10571499726.70			
G	1	112 DCIS	40	39.75	0.43	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G	2	112 DCIS	39.2633			0.00			0.00			10571499726.70			
G	3	112 DCIS	40			0.00			0.00			10571499726.70			
G	4	112 IDC	28.0929	28.16	0.06	1.11	1.06	0.04	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G	5	112 IDC	28.1721			1.05			0.00			10571499726.70			
G	6	112 IDC	28.2131			1.03			0.00			10571499726.70			
G	7	121 N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G	8	121 N	40			0.00			0.00			10571499726.70			
G	9	121 N	40			0.00			0.00			10571499726.70			
G	10	121 DCIS	38.4431	38.40	0.32	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G	11	121 DCIS	38.062			0.00			0.00			10571499726.70			
G	12	121 DCIS	38.6966			0.00			0.00			10571499726.70			
H	1	121 IDC	27.7402	27.79	0.11	1.39	1.35	0.09	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H	2	121 IDC	27.8157			1.24			0.00			10571499726.70			
H	3	121 IDC	27.7232			1.41			0.00			10571499726.70			
H	4	122 N	33.3884	33.56	0.32	0.04	0.03	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H	5	122 N	33.9338			0.03			0.00			10571499726.70			
H	6	122 N	33.3679			0.04			0.00			10571499726.70			
H	7	122 DCIS	39.4549	39.82	0.31	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H	8	122 DCIS	40			0.00			0.00			10571499726.70			
H	9	122 DCIS	40			0.00			0.00			10571499726.70			
H	10	122 IDC	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H	11	122 IDC	40			0.00			0.00			10571499726.70			
H	12	122 IDC	40			0.00			0.00			10571499726.70			

MMP1 #4

			TARGET						Normalizer (Cyclophilin, 18S)						
		Sample	Ct	Mean Ct	St. Dev.	ng	Avg. ng	St.Dev. ng	Ct	Mean Ct	St. Dev.	ng	Avg. ng	St.Dev. ng	
A	1	Stock 1:4	20.1107	20.10	0.05	190.47	191.63	5.63	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A	2	Stock 1:4	20.1424			186.68			0.00			#DIV/0!			
A	3	Stock 1:4	20.0516			197.75			0.00			#DIV/0!			
A	4	Stock 1:16	22.0372	22.16	0.11	56.08	51.86	3.75	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A	5	Stock 1:16	22.1979			50.64			0.00			#DIV/0!			
A	6	Stock 1:16	22.2536			48.88			0.00			#DIV/0!			
A	7	Stock 1:64	24.2258	24.31	0.17	13.98	13.31	1.36	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A	8	Stock 1:64	24.4999			11.75			0.00			#DIV/0!			
A	9	Stock 1:64	24.2			14.21			0.00			#DIV/0!			
A	10	Stock 1:256	26.6733	26.67	0.02	2.96	2.97	0.04	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A	11	Stock 1:256	26.6441			3.01			0.00			#DIV/0!			
A	12	Stock 1:256	26.6826			2.94			0.00			#DIV/0!			
B	1	170N	37.665	37.56	0.10		0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	35.01
B	2	170N	37.4808			0.00			0.00			10571499726.70			
B	3	170N	37.5263			0.00			0.00			10571499726.70			
B	4	170DCIS	40	39.86	0.58	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
B	5	170DCIS	40			0.00			0.00			10571499726.70			
B	6	170DCIS	38.9895			0.00			0.00			10571499726.70			
B	7	170IDC	27.1609	27.13	0.06		2.24	0.10	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	38.13
B	8	170IDC	27.1648			2.16			0.00			10571499726.70			
B	9	170IDC	27.0629			2.31			0.00			10571499726.70			
B	10	173N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
B	11	173N	40			0.00			0.00			10571499726.70			
B	12	173N	40						0.00			10571499726.70			37.28
C	1	173DCIS	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C	2	173DCIS	40			0.00			0.00			10571499726.70			
C	3	173DCIS	40			0.00			0.00			10571499726.70			
C	4	173IDC	38.7628	39.27	0.46	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C	5	173IDC	39.6507			0.00			0.00			10571499726.70			
C	6	173IDC	39.3906			0.00			0.00			10571499726.70			
C	7	178N	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C	8	178N	40			0.00			0.00			10571499726.70			
C	9	178N	40			0.00			0.00			10571499726.70			
C	10	178DCIS	40	39.92	0.14	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	new
C	11	178DCIS	40			0.00			0.00			10571499726.70			
C	12	178DCIS	39.7661			0.00			0.00			10571499726.70			
D	1	178IDC	27.9588	27.86	0.17	1.31	1.40	0.15	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D	2	178IDC	27.9624			1.30			0.00			10571499726.70			
D	3	178IDC	27.6869			1.57			0.00			10571499726.70			
D	4	179N	39.2317	39.75	0.43	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D	5	179N	40			0.00			0.00			10571499726.70			
D	6	179N	40			0.00			0.00			10571499726.70			
D	7	179DCIS	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	new
D	8	179DCIS	40			0.00			0.00			10571499726.70			
D	9	179DCIS	40			0.00			0.00			10571499726.70			
D	10	179IDC	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D	11	179IDC	40			0.00			0.00			10571499726.70			
D	12	179IDC	40			0.00			0.00			10571499726.70			
E	1	11N	35.4536	35.65	0.22	0.01	0.01	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E	2	11N	35.8063			0.01			0.00			10571499726.70			
E	3	11N	35.8863			0.01			0.00			10571499726.70			
E	4	11DCIS	40	39.80	0.35	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E	5	11DCIS	39.3992			0.00			0.00			10571499726.70			
E	6	11DCIS	40						0.00			10571499726.70			34.11
E	7	12N	40	39.75	0.43	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E	8	12N	39.257			0.00			0.00			10571499726.70			
E	9	12N	40			0.00			0.00			10571499726.70			
E	10	12DCIS	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E	11	12DCIS	40						0.00			10571499726.70			33.47
E	12	12DCIS	40			0.00			0.00			10571499726.70			
F	1	191N	32.0882	32.13	0.06	0.10	0.09	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F	2	191N	32.1942			0.09			0.00			10571499726.70			
F	3	191N	32.1086			0.09			0.00			10571499726.70			
F	4	191ADH	32.9583	33.23	0.24	0.05	0.05	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F	5	191ADH	33.4205			0.04			0.00			10571499726.70			
F	6	191ADH	33.3225			0.04			0.00			10571499726.70			
F	7	191DCIS	29.2734	29.31	0.11	0.57	0.55	0.04	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F	8	191DCIS	29.2344			0.58			0.00			10571499726.70			
F	9	191DCIS	29.4367			0.51			0.00			10571499726.70			
F	10	193N	39.1384	39.71	0.50	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F	11	193N	40			0.00			0.00			10571499726.70			
F	12	193N	40			0.00			0.00			10571499726.70			
G	1	193ADH	37.5042	37.27	0.41	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G	2	193ADH	37.5168			0.00			0.00			10571499726.70			
G	3	193ADH	36.8008			0.00			0.00			10571499726.70			
G	4	193DCIS	28.8513	28.77	0.07	0.74	0.78	0.03	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G	5	193DCIS	28.7464			0.79			0.00			10571499726.70			
G	6	193DCIS	28.7167			0.81			0.00			10571499726.70			
G	7	193IDC	29.4225	29.33	0.10	0.52	0.55	0.03	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G	8	193IDC	29.2263			0.58			0.00			10571499726.70			
G	9	193IDC	29.3479			0.54			0.00			10571499726.70			
G	10	196N	33.2597	33.24	0.05	0.05	0.05	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G	11	196N	33.2754			0.04			0.00			10571499726.70			
G	12	196N	33.1796			0.05			0.00			10571499726.70			
H	1	198DCIS	37.1448	37.57	0.42	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H	2	198DCIS	37.9875			0.00			0.00			10571499726.70			
H	3	198DCIS	37.5752			0.00			0.00			10571499726.70			
H	4	198IDC	29.82	29.97	0.13	0.40	0.37	0.03	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H	5	198IDC	30.0555			0.35			0.00			10571499726.70			
H	6	198IDC	30.0372			0.35			0.00			10571499726.70			
H	7	213N	36.5551	36.64	0.33	0.01	0.01	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H	8	213N	36.3543			0.01			0.00			10571499726.70			
H	9	213N	36.8978			0.00			0.00			10571499726.70			
H	10	213ADH	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H	11	213ADH	40			0.00			0.00			10571499726.70			
H	12	213ADH	40			0.00			0.00			10571499726.70			

MMP1 #5

			TARGET						Normalizer (Cyclophilin, 18S)							
		Sample	Ct	Mean Ct	St. Dev.	ng	Avg. ng	St.Dev. ng	Ct	Mean Ct	St. Dev.	ng	Avg. ng	St.Dev. ng	
A	1	Stock 1:4	20.2949	20.21	0.08	184.09	195.06	10.46	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A	2	Stock 1:4	20.196			196.16			0.00			#DIV/0!			
A	3	Stock 1:4	20.1276			204.93			0.00			#DIV/0!			
A	4	Stock 1:16	22.3333	22.29	0.06	49.74	51.21	1.94	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A	5	Stock 1:16	22.2223			53.42			0.00			#DIV/0!			
A	6	Stock 1:16	22.3105			50.48			0.00			#DIV/0!			
A	7	Stock 1:64	24.3914	24.44	0.08	13.27	12.90	0.61	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A	8	Stock 1:64	24.5233			12.19			0.00			#DIV/0!			
A	9	Stock 1:64	24.395			13.24			0.00			#DIV/0!			
A	10	Stock 1:256	26.7071	26.69	0.13	3.00	3.04	0.25	0.00	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!	
A	11	Stock 1:256	26.5522			3.32			0.00			#DIV/0!			
A	12	Stock 1:256	26.8067			2.82			0.00			#DIV/0!			
B	1	8 DCIS	40	40.00	0.00		0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	35.01
B	2	8 DCIS	40			0.00			0.00			10571499726.70			
B	3	8 DCIS	40			0.00			0.00			10571499726.70			
B	4	8 IDC	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
B	5	8 IDC	40			0.00			0.00			10571499726.70			
B	6	8 IDC	40			0.00			0.00			10571499726.70			
B	7	22 ADH	40	40.00	0.00		0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	38.13
B	8	22 ADH	40			0.00			0.00			10571499726.70			
B	9	22 ADH	40			0.00			0.00			10571499726.70			
B	10	22 DCIS	40	39.97	0.06	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
B	11	22 DCIS	40			0.00			0.00			10571499726.70			
B	12	22 DCIS	39.9013						0.00			10571499726.70			37.28
C	1	25 DCIS	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C	2	25 DCIS	40			0.00			0.00			10571499726.70			
C	3	25 DCIS	40			0.00			0.00			10571499726.70			
C	4	25 IDC	38.6057	39.54	0.80	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C	5	25 IDC	40			0.00			0.00			10571499726.70			
C	6	25 IDC	40			0.00			0.00			10571499726.70			
C	7	40 N	40	39.56	0.76	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C	8	40 N	40			0.00			0.00			10571499726.70			
C	9	40 N	38.6839			0.00			0.00			10571499726.70			
C	10	40 LCIS	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
C	11	40 LCIS	40			0.00			0.00			10571499726.70			
C	12	40 LCIS	40			0.00			0.00			10571499726.70			
D	1	78-1 MPR	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D	2	78-1 MPR	40			0.00			0.00			10571499726.70			
D	3	78-1 MPR	40			0.00			0.00			10571499726.70			
D	4	78-3 MPR	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D	5	78-3 MPR	40			0.00			0.00			10571499726.70			
D	6	78-3 MPR	40			0.00			0.00			10571499726.70			
D	7	97 DCIS	22.1868	22.19	0.02	54.65	54.46	0.62	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D	8	97 DCIS	22.2119			53.78			0.00			10571499726.70			
D	9	97 DCIS	22.1779			54.96			0.00			10571499726.70			
D	10	210 N	39.414	38.73	0.60	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
D	11	210 N	38.4893			0.00			0.00			10571499726.70			
D	12	210 N	38.2814			0.00			0.00			10571499726.70			
E	1	210 ADH	29.5663	29.49	0.08	0.48	0.50	0.02	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E	2	210 ADH	29.4127			0.53			0.00			10571499726.70			
E	3	210 ADH	29.5032			0.50			0.00			10571499726.70			
E	4	210 DCIS	39.6616	38.68	0.98	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E	5	210 DCIS	37.7102			0.00			0.00			10571499726.70			
E	6	210 DCIS	38.6799						0.00			10571499726.70			34.11
E	7	215 MPR	33.1982	33.20	0.12	0.05	0.05	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E	8	215 MPR	33.3238			0.04			0.00			10571499726.70			
E	9	215 MPR	33.0803			0.05			0.00			10571499726.70			
E	10	NTC	40	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
E	11	NTC	40						0.00			10571499726.70			
E	12	NTC	40			0.00			0.00			10571499726.70			33.47
F	1	MCF-7 Unamp	37.2432	37.73	0.74	0.00	0.00	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F	2	MCF-7 Unamp	37.3727			0.00			0.00			10571499726.70			
F	3	MCF-7 Unamp	38.5823			0.00			0.00			10571499726.70			
F	4	Daudi Unamp	35.5411	35.52	0.13	0.01	0.01	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F	5	Daudi Unamp	35.3724			0.01			0.00			10571499726.70			
F	6	Daudi Unamp	35.637			0.01			0.00			10571499726.70			
F	7	MCF-7 Amp	35.8603	35.66	0.25	0.01	0.01	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F	8	MCF-7 Amp	35.3801			0.01			0.00			10571499726.70			
F	9	MCF-7 Amp	35.7501			0.01			0.00			10571499726.70			
F	10	Daudi Amp	33.2153	33.03	0.22	0.05	0.05	0.01	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
F	11	Daudi Amp	33.0997			0.05			0.00			10571499726.70			
F	12	Daudi Amp	32.7861			0.06			0.00			10571499726.70			
G	1	0		#DIV/0!	#DIV/0!	83779915.69	83779915.69	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G	2	0				83779915.69			0.00			10571499726.70			
G	3	0				83779915.69			0.00			10571499726.70			
G	4	0		#DIV/0!	#DIV/0!	83779915.69	83779915.69	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G	5	0				83779915.69			0.00			10571499726.70			
G	6	0				83779915.69			0.00			10571499726.70			
G	7	0		#DIV/0!	#DIV/0!	83779915.69	83779915.69	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G	8	0				83779915.69			0.00			10571499726.70			
G	9	0				83779915.69			0.00			10571499726.70			
G	10	0		#DIV/0!	#DIV/0!	83779915.69	83779915.69	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
G	11	0				83779915.69			0.00			10571499726.70			
G	12	0				83779915.69			0.00			10571499726.70			
H	1	0		#DIV/0!	#DIV/0!	83779915.69	83779915.69	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H	2	0				83779915.69			0.00			10571499726.70			
H	3	0				83779915.69			0.00			10571499726.70			
H	4	0		#DIV/0!	#DIV/0!	83779915.69	83779915.69	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H	5	0				83779915.69			0.00			10571499726.70			
H	6	0				83779915.69			0.00			10571499726.70			
H	7	0		#DIV/0!	#DIV/0!	83779915.69	83779915.69	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H	8	0				83779915.69			0.00			10571499726.70			
H	9	0				83779915.69			0.00			10571499726.70			
H	10	0		#DIV/0!	#DIV/0!	83779915.69	83779915.69	0.00	0.00	0.00	0.00	10571499726.70	10571499726.70	0.00	
H	11	0				83779915.69			0.00			10571499726.70			
H	12	0				83779915.69			0.00			10571499726.70			

Case ID	Stages captured	Age	ER	PR	HER2	Node
8	DCIS (III),IDC (III)	48	Pos	Pos	Pos	Pos
14	N,DCIS (I),IDC (I)	44	Pos	Pos	ND	Pos
22	ADH,DCIS (I)	44	ND	ND	ND	Pos
25	DCIS (I),IDC (II)	81	Pos	Neg	ND	ND
30	N,DCIS (III),IDC (III)	47	Neg	Neg	Neg	Pos
41	N,DCIS (II),IDC (II)	55	Pos	Pos	ND	Neg
43	N,DCIS (II),IDC (II)	53	Pos	Neg	Neg	Pos
44	N,DCIS (III),IDC (III)	28	Pos	Pos	Neg	Neg
45	N,DCIS (I)	36	Pos	Neg	Neg	Neg
57	N,ADH,DCIS (I)	34	ND	ND	ND	Neg
65	N,DCIS (III),IDC (III)	39	Pos	Pos	Neg	Neg
78	MPR	46	ND	ND	ND	ND
79	N,ADH,DCIS (I),IDC (I)	54	Pos	Pos	Neg	Pos
88	N,DCIS (III),IDC (III)	35	Pos	Pos	ND	Pos
95	MPR	16	ND	ND	ND	ND
96	N,DCIS (III),IDC (III)	31	Neg	Neg	Neg	Pos
97	DCIS (III),IDC (III)	79	Neg	Neg	Pos	Pos
102	N,DCIS (I),IDC (I)	55	Pos	Neg	Neg	Pos
112	N,DCIS (III),IDC (III)	31	Neg	Pos	Neg	Pos
121	N,DCIS (II),IDC (II)	45	Pos	Pos	Pos	Pos
130	N,DCIS (II),IDC (II)	54	Pos	Pos	Neg	Pos
131	N,ADH,DCIS (II),IDC (II)	37	Pos	Pos	Pos	Pos
133	N,DCIS (III),IDC (III)	44	Neg	Neg	Pos	Pos
148	N,DCIS (II),IDC (II)	42	Pos	Pos	Neg	Pos
152	N,DCIS (III)	55	ND	ND	ND	Neg
153	N,IDC (I)	46	Pos	Pos	Pos	Pos
169	N,DCIS (II),IDC (II)	34	Pos	Pos	Neg	Pos
170	N,DCIS (II),IDC (II)	44	Pos	Pos		Pos
173	N,DCIS (I),IDC (I)	52	Pos	Pos	Neg	Neg
178	N,DCIS (III),IDC (III)	43	Pos	Pos	Pos	Pos
179	N,DCIS (III),IDC (III)	37	Neg	Neg	Pos-FISH	Pos
180	N,ADH,DCIS (I),IDC (I)	46	Pos	Pos		Pos
183	N,DCIS (II)	46	ND	ND	ND	Pos
191	N,ADH,DCIS (II)	43	ND	ND	ND	ND
193	N,ADH,DCIS (I),IDC (I)	45	Pos	Pos	Neg	Pos
198	N,DCIS (II),IDC (II)	30	Pos	Pos		Neg
210	N,ADH,DCIS (I)	62	ND	ND	ND	Neg
213	N,ADH	45	ND	ND	ND	Neg
215	MPR	30	ND	ND	ND	ND

APPENDIX II: FOLD INCREASE IN MMP-1 EXPRESSION IN GRADE I, II AND III BREAST CANCERS

Exponent	Grade	Fold increase
5	I	32.0
2	I	4.0
6	I	64.0
5	I	32.0
6	I	64.0
0.5	I	1.4
11	I	2048.0
7	II	128.0
10	II	1024.0
12	II	4096.0
15	II	32768.0
10	II	1024.0
3	II	8.0
3	II	8.0
8	II	256.0
3	II	8.0
15	III	32768.0
7.5	III	181.0
18	III	262144.0
7	III	128.0
2	III	4.0
12	III	4096.0
10	III	1024.0
11	III	2048.0
12	III	4096.0

Average

Grade	Fold Increase	Stand Dev	SEM
I	320.8	762.0	288.0
II	4368.9	10729.1	3576.4
III	34054.3	86164.7	28721.6

Values excluding outliers (+/- 2*st dev)

Grade	Fold Increase	Stand Dev
I	32.9	27.43212
II	819.0	1393.372
III	5543.1	11127.69

Manuscripts/Abstracts

For DOD BRCP meeting, September 2002:

GENETIC ANALYSIS OF A SINGLE NUCLEOTIDE POLYMORPHISM IN THE MATRIX METALLOPROTEINASE 1 (MMP-1) PROMOTER IN BREAST CANCER

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Death from breast cancer results from tumor metastasis, and the *sine qua non* of metastasis is degradation of the extracellular matrix, a process that is mediated primarily by Matrix Metalloproteinases (MMPs). Destruction of the interstitial collagens, types I and III, is a necessary part of the process, since these collagens comprise nearly 30% of body protein and the connective tissues through which tumor cells must travel during invasion. Of the three interstitial collagenases that can contribute to invasion, MMP-1 (collagenase-1) is the most ubiquitously expressed and thus, has the greatest potential for facilitating tumor invasion.

We have found a single nucleotide polymorphism (SNP) in the MMP-1 promoter that enhances transcription of this gene in tumor cells and in normal stromal cells, thereby potentially facilitating cancer progression by more aggressive degradation of the interstitial matrix. The SNP is located at -1607 bp in the MMP-1 promoter, where an additional guanine (G) creates a binding site (5'-AGGA-3') for members of the Ets family of transcription factors, and the absence of the G (5'-AGA-3') lacks the binding site. The frequency of this SNP in the population is 25% =1 G, 25% =2 G, and 50% = heterozygous. The 2G allele has been associated with increased incidence or progression in five cancers: ovarian, endometrial, melanoma, colon and lung, and this study investigates its potential role in breast cancer.

We first examined the association of the 2G allele with the incidence of breast cancer. The 1G/2G genotype of 157 women was evaluated by PCR of DNA obtained by buccal swabs (Cancer Epidemiology, Biomarkers, and Prevention. 10: 687, 2001). Of these, 82 were invasive breast cancer cases representing ductal, lobular, and ductal with a lobular component, and 75 were from normal controls. The genotypes for women with cancer were: 29% = 1G homozygous, 21% = 2G homozygous and 50% = 1G/2G heterozygous, and the types for the control women were: 27% =1G homozygous, 27% = 2G homozygous and 47% = 1G/2G heterozygous. Thus, there appears to be no link between the 2G genotype and the incidence of breast cancer.

We next investigated the association between the 2G allele and breast cancer metastasis and progression. We have begun our analysis with invasive ductal carcinoma, since this is the most common type of breast cancer. Based on our previous experiences with metastatic melanoma (Am. J. Pathol. 158: 691, 2001), we genotyped DNA from 58 patients with overt metastatic breast cancer and found no deviation from control values, suggesting that the 2G allele does not favor metastasis. We have also analyzed tumor tissue from the heterozygotes for Loss of Heterozygosity (LOH) at the 11q 22-23 locus, a common site for LOH in breast cancer and the location of the MMP-1 gene, since we hypothesized that retention of the 2G allele after LOH provided tumors with an advantage for progression. We used our ^{32}P PCR assay with overlapping sets of primers (82 bp or 72 bp) to amplify DNA from the tumor tissue. Of 24 heterozygotes, we observed LOH in only 5, with retention of the 2G allele in 3 cases. Thus, additional samples need to be analyzed before we can conclude that the presence of the 2G allele in the MMP-1 promoter signifies greater invasive potential of the tumor. This allelic variation may be a meaningful genetic marker that can help identify those women at higher risk for invasive/metastatic disease, and may have important implications for the diagnosis and treatment of certain types of breast cancer.